Narumi NAKATO* & Shunsuke SERIZAWA**: **Chromosome numbers of the genus *Microlepia* in Japan with description of a new hybrid**

中藤成実*・芹沢俊介**: 邦産フモトシダ属植物の染色体数と新雑種

Microlepia is a genus of the dennstaedtioid ferns. It consists of about 50 species, and most of the species are distributed in Southeast Asia. A considerable amount of cytological works has been made on this genus, and the chromosome number has been examined for 13 taxa (Manton & Sledge, 1954; Manton, 1954, 1958, 1959; Mehra & Khanna, 1959; Abraham et al., 1962; Kurita, 1963, 1967a, 1967b, 1972; Holttum & Roy, 1965; Mitui, 1965, 1968, 1973, 1975, 1976; Sorsa in Fabbri, 1965; Kuriachen, 1967; Sasamoto, 1970; Roy et al., 1971; Tsai, 1972; Kawakami, 1979). Most of these works reported the chromosome number of this genus to be $n=43$ or its multiples, except for the observations of $n=88$ in *M. speluncae* by Abraham et al. (1962), $2n=160$ in *M. pseudostrigosa* by Kurita (1963) and $2n=84$ in *M. strigosa* by Holttum & Roy (1965) and Kawakami (1979), which suggest the basic number 44, 40 and 42, respectively.

The authors have reexamined five Japanese taxa of this genus and found four kinds of the somatic chromosome number, $2n=84$, 126, 168 and 252. They suggest the basic chromosome number $x=42$. In the present paper, the results are reported with description of a new hybrid.

Materials and methods A total of 27 individuals belonging to five taxa were collected from the habitats and their localities are listed in Table 1 with the cytological results. Mitotic metaphase chromosomes were observed in root tip cells pretreated with 0.002M 8-hydroxyquinoline solution for 3 hours. The root tips were fixed in 45% acetic acid for 10 minutes, immersed in a mixture of 1N HCl and 45% acetic acid (2: 1) at 60°C for 2 minutes and squashed in 2% aceto-orcein. All voucher specimens are preserved in the herbarium of Aichi Kyoiku University.

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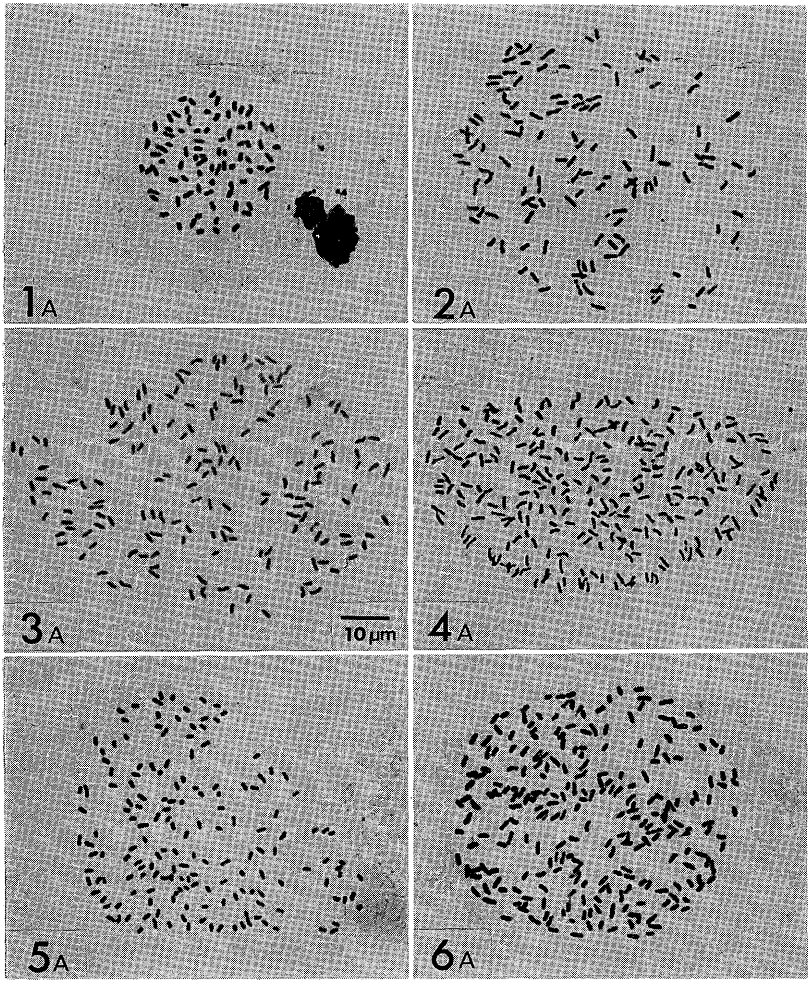
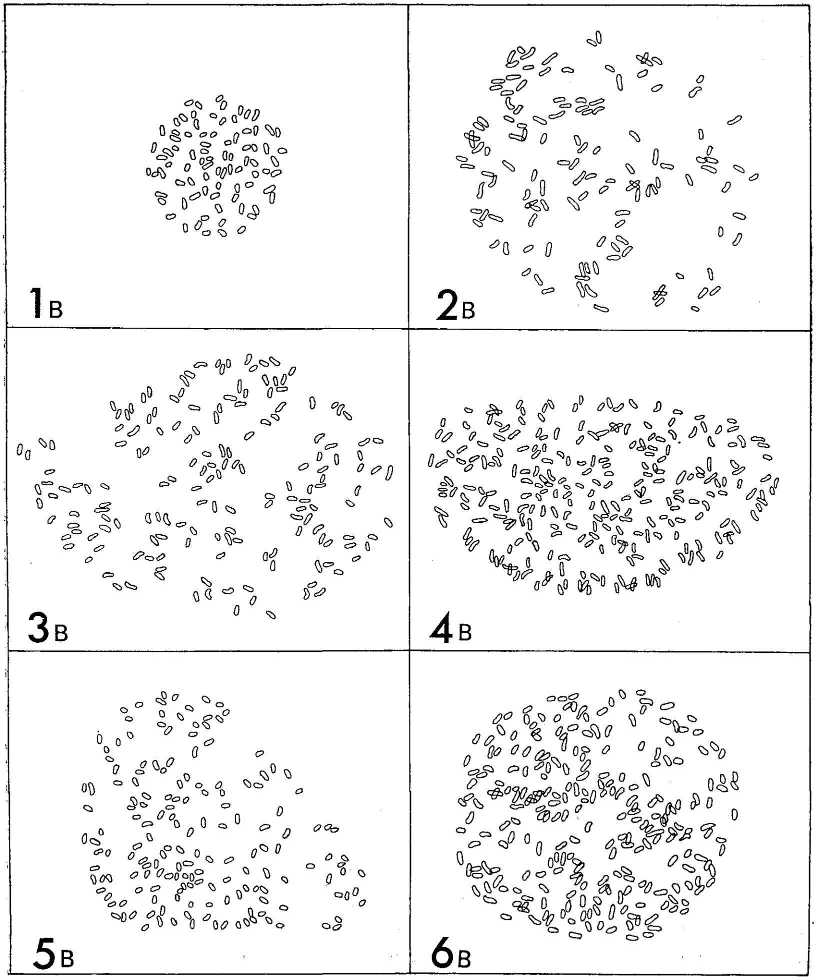
Observations and discussion 1. *M. strigosa* (Thunb.) Pr. This species is widely distributed in tropical Asia, ranging north to Japan. Mitui (1965, 1968, 1973, 1976) and Sasamoto (1970) reported the chromosome number n=43



Figs. 1A-6A. Photomicrographs of somatic chromosomes. 1A. *M. strigosa*. 2n=84 (Nakato no. 452). 2A. *M.×austroizuensis*, 2n=126 (no. 643). 3A. *M. marginata*, 2n=168 (no. 555). 4A. *M. marginata*, 2n=252 (no. 366). 5A. *M.×bipinnata*, 2n=168 (no. 430). 6A. *M. pseudo-strigosa*, 2n=252 (no. 632).

and Kurita (1972) observed 2n=86 for the Japanese plants. Holttum & Roy (1965), however, reported 2n=84 for the plant from New Guinea. Kawakami (1979) also reported 2n=84=10 sm+32 st+42 t for the Japanese plants. In the present study, five plants from four localities were examined and the somatic chromosome number 2n=84 was observed in all of these materials (Figs. 1A &



Figs. 1B-6B. Explanatory diagrams of 1A-6A.

1B). They are supposed to be diploid based on x=42.

2. *M.* ×*austroizuensis* Nakato et Serizawa.[1] There is a large colony of problematical *Microlepia* at Nagatsuro of Izu Peninsula. Two plants collected from this colony showed 2n=126 chromosomes (Figs. 2A & 2B) and irregular formation of spores. They are supposed to be triploid based on x=42.

This plant was first noticed by Kurata (1962). He refferred the specimens collected from this colony to his new species *M. izu-peninsulae,* which was later reduced to a synonym of *M. sino-strigosa* (Serizawa, 1972). Kurata (1979) also illustrated a specimen of this plant as "*M. izupeninsulae*". However, this fern differs from *M. sino-strigosa* in more numerous pinnae, small and more numerous pinnulae and not reniform but half-cup-shaped indusia. These characters of this fern are somewhat similar to those of *M. strigosa.* Namely this fern is morphologically intermediate between *M. sino-strigosa* and *M. strigosa*. As to the chromosome number of *M. sino-strigosa,* the numbers of tetraploid level have been reported by Kurita (1967b, n=84-86), Sasamoto (1970, n=86) and Mitui (1975, n=86). The authors, therefore, consider the plant at Nagatsuro (3x) to be a natural hybrid between *M. sino-strigosa* (4x) and *M. strigosa* (2x), and named it *M.* ×*austroizuensis.*

3. *M. marginata* (Panzer) C. Chr. This species is distributed in East Asia, and common in the southern parts of Honshu, Shikoku and Kyushu, Japan. The following chromosome counts were previously reported for this species; 2n=c. 170 (Kurita, 1967a), n=c. 86 (Mitui, 1968) and n=86 (Sasamoto, 1970; Tsai, 1972). In the present study, the authors examined 16 materials from 9 localities and found two different cytotypes, i. e. 2n=168 (Figs. 3A & 3B, probably

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[1] **Microlepia ×austroizuensis** Nakato et Serizawa, hybr. nov.—*M. izu-peninsulae* auct. non Kurata: Kurata, Ill. Pter. Jap. 1: 137 (1979).

Microlepia sino-strigosa Ching × *Microlepia strigosa* (Thunb.) Pr.

Ab anteriore pinnis multioribus, pinnulis minoribus multioribus, indusiis semicupuliformibus vel late semicupuliformibus differt; a posteriore lamina minus dissecta, soris intramarginalibus, indusiis saepe latioribus differt.

Nom. Jap. Nise-odorikokaguma, nov.

Hab. Honshu. Pref. Shizuoka: Nagatsuro, Minamiizu-machi (S. Serizawa no. 5525, Dec. 27, 1967, AICH); ibid. (N. Nakato no. 643, Mar. 21, 1979, AICH—holotype, 2n=126); ibid. (N. Nakato no. 640, Mar. 21, 1979, AICH). Kyushu. Pref. Kumamoto: near Tsurunoyu, Sakamoto-mura (S. Serizawa no. 15232, Oct. 24, 1971, AICH). Pref. Kagoshima: Hinotani, Mt. Shibi-san, Izumi-shi (S. Serizawa no. 30214, Aug. 1, 1979, AICH).

tetraploid of x=42) and 2n=252 (Figs. 4A & 4B, probably hexaploid of x=42).

The tetraploid cytotype was found at all localities examined in this study, but the hexaploid was detected only from one locality (Table 1). The tetraploid cytotype seems to be more widely distributed than the hexaploid in Japan. As far as the plants used in the present study were concerned, there was a difference between the two cytotypes in the width of lateral pinnae. The hexaploid plants were characterized by broader pinnae (2.5-3.5 cm wide in the largest pinnae of fertile leaves), while the tetrapleid plants had narrower ones (1.2-2.3 cm wide). This difference, however, seems to be too unreliable to separate taxa.

Tab. 1. Summary of chromosome counts.

Taxa	Localities	Number of plants examined	Chromosome numbers	Voucher specimens (Nakoto's no.)
M. strigosa	Suzaki, Shizuoka Pref.	2	2n=84	450, 452
〃	Owase, Mie Pref.	1	2n=84	670
〃	Nachi, Wakayama Pref.	1	2n=84	706
〃	Isl. Yakushima, Kagoshima Pref.	1	2n=84	669
M. × *austroizuensis*	Nagatsuro, Shizuoka Pref.	2	2n=126	640, 643
M. marginata	Takaosan, Tokyo Pref.	3	2n=168	624, 626, 627
〃	Jinmuji, Kanagawa Pref.	1	2n=168	529
〃	〃	2	2n=252	366, 525
〃	Onabe, Shizuoka Pref.	3	2n=168	634, 635, 636
〃	Nagatsuro, Suizuoka Pref.	1	2n=168	644
〃	Ashikubo, Shizuoka Pref.	1	2n=168	480
〃	Akame-kyo, Mie Pref.	1	2n=168	555
〃	Kyoto, Kyoto Pref.	1	2n=168	541
〃	Nara, Nara Pref.	1	2n=168	537
〃	Aburayama, Fukuoka Pref.	2	2n=168	456, 463
M. × *bipinnata*	Shimoda, Shizuoka Pref.	2	2n=168	430, 433
M. pseudostrigosa	Jinmuji, Kanagawa Pref.	1	2n=252	367
〃	Onabe, Shizuoka Pref.	1	2n=252	632

4. *M.* ×*bipinnata* (Makino) Shimura. This fern has usually been regarded as a bipinnate variety of *M. marginata*. Mitui (1968) reported the chromosome count n=c. 126/2 and the occurence of many bivalents and univalents at meiosis, and considered this "variety" to be a hybrid between *M. marginata* (4x) and *M. strigosa* (2x). The two plants used in the present study were morphologically referable to *M.* ×*bipinnata*, but they showed the chromosome number 2n=168 and are supposed to be tetraploid based on x=42 (Figs. 5A & 5B). Thus, this fern seems to have two cytotypes, triploid and tetraploid.

The tetraploid plants examined in this study were not normal in spore formation. The sporangia ceased their developments on the way to mature, and the spores are lacking or, if present, irregular in shape and size. Our plants may be a hybrid between *M. marginata* (6x) and *M. strigosa* (2x), or *M. marginata* (4x) and *M. sino-strigosa* (4x).

5. *M. pseudo-strigosa* Makino. This species is endemic to the Pacific side of central Honshu of Japan. The chromosome counts 2n=160 (Kurita, 1963), n=126-129 (Sasamoto, 1970) and n=129 (Mitui, 1975) were previously reported for this species. Two plants examined in this study showed 2n=252 chromosomes (Figs. 6A & 6B), which were considered to be hexaploid based on x=42.

Problem of the basic chromosome number Since Manton & Sledge (1954) reported the haploid chromosome numbers of *M. platyphylla* and *M. speluncae* from Sri Lanka (Ceylon) to be n=43 and n=86 respectively, x=43 has generally been accepted as the basic chromosome number of the genus *Microlepia*. However, new additional numbers such as x=40, 42 and 44 were reported in the cytological studies on *M. pseudo-strigosa* (Kurita, 1963), *M. strigosa* (Holttum & Roy, 1965; Kawakami, 1979) and *M. speluncae* (Abraham et al., 1962), respectively. In the present study, the authors examined the chromosome numbers of 27 materials belonging to five taxa. The numbers observed were 2n=84, 126, 168 and 252, and the basic number was supposed to be x=42. This basic number agrees with the observations of Holttum & Roy (1965) and Kawakami (1979), but differs from those of the other authors.

Two alternative explanations may be possible for the disagreement between the present and previous counts. One is that it was caused by the miss counting of chromosomes in the previous studies. Most of the previous observations were made on meiotic plates. Mehra & Khanna (1959) stated that the chromosomes and spore mother cells of Dennstaedtiaceae were small and it was difficult to obtain clear nuclear plates of meiosis. Mitui (1968) also mentioned that the distinction between bivalents and univalents of *M. marginata* var. *bipinnata* (=*M.* ×*bipinnata*) was rather difficult because of their small sizes. Kurita (1972) reported 2n=86 in the mitotic plates of *M. strigosa*, but his observation was made on the prophase plates in which the accurate count of chromosomes seemed to be rather difficult. From these circumstances, it may be possible to consider that the previous counts suggesting x=43 were erroneous.

The other explanation is that there are two different basic chromosome numbers, x=42 and 43, in every taxon examined in this study. The authors could not observed the chromosome numbers multiples of 43 in any somatic cells, but the possibility of this case cannot be completely eliminated. At any rate, it is safely concluded from the present study that there widely exists the basic chromosome number x=42 in the Japanese members of *Microlepia*. The basic number x=43 should be verified by accurate counts on mitosis.

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邦産フモトシダ属植物3種2雑種の体細胞染色体数を算定した。結果は，イシカグマが 2n=84，フモトシダが 2n=168 及び 2n=252，フモトカグマが 2n=252，ニセオドリコカグマ（新称，オドリコカグマ×イシカグマ）が 2n=126，クジャクフモトシダが 2n=168 であり，染色体基本数は x=42 と推察された。従来，邦産のフモトシダ属では，主として x=43 が報告されていたが，本当に x=43 の系統があるのかどうか，再検討を要する。各々の種類についてみると，フモトシダに種内倍数体（4x・6x）があること，従来3x のみ知られていたクジャクフモトシダに4x も存在すること等が明らかになった。

□池上義信（監修）・石沢進（編集）：**新潟県植物分布図集　第1集**［Y. Ikegami (comp.), S. Ishizawa (ed.): Distribution maps of vascular plants in Niigata Prefecture (Japan) Vol. 1］178 pp., 50 maps, 12 pls. 1980. 植物同好じねんじょ会（新潟県小千谷市山寺，関省吾方）. ¥3,500.　新潟県におけるシダ類および種子植物の分布を正確に記録し，同県産植物の集大成を目的としたもので，正式印刷された府県単位の分布図集の嚆矢である。（本書以前には青森県生物学会（1967～'73年）の作ったとう写版のものがある）。約100万分の1の等高線入りの淡色地図に，金井方式で記録された50種の分布点が黒丸で示され，美事な出来ばえである。分布図ごとに資料標本のくわしいデータリスト，関係文献およびノートが付いている。植物写真がプレートに示され，巻末には石沢による県内の分布型20型が概説されている。大縮尺の分布では日本全図とは異なった発見があり興味深い。たとえばヒトリシズカ，マルバハギ，クマノミズキなどである。これらのデータはカード方式で整理されており，綴じ本の他に加除式のものも作られている。基礎のしっかりした分布図の作成は，完全な植物誌にとって不可欠なものだが，多年にわたる大変な努力を要するため，どこでも実行にかかれなかった。監修編集の両氏をはじめ，関省吾，伊藤至氏ら44名の同志の熱意に敬意を表すると共に，その完成を期待したい。わが国のローカルフロラ研究も，府県単位の分布図を作る段階に入り，今後これを参考に同様な企画が生れると思うので，以下の点を希望する。標本データの引用は略さずに記してほしい。本書では採集者略号が意外とわかりにくい。また分布点と標本の対応がつけられないのも不便である。個人所蔵の標本が多いが，これらは将来にわたって参照できるよう，しかるべき公的機関に収蔵することを考えてもらいたい。地域的に精疎があるのはやむを得ないが，なるべく多くの人が参加できるような形に発展させてほしい。また大学や研究所所蔵の標本も積極的にとり入れれば，一層充実したものとなるだろう。（前川文夫，原寛，金井弘夫）